EXPERIMENTS WITH KENDALL'S MEDIUM[1]

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Received for publication, May 12, 1932

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Recently, Kendall (1931) has described a culture medium which is nearly free of altered protein and is peptone-poor. In this medium he was able to cultivate filterable cocci from the blood of influenza patients, individuals ill with the common cold, rabies virus and vaccine virus. Typhoid bacilli and streptococci were converted into filterable organisms in this medium and from staphylococcus bacteriophage, staphylococci were recovered. The apparent ease with which these transformations were effected has excited widespread curiosity and has led to this repetition of Kendall's work. Recently, Craig and Johns (1932) have attempted to repeat Kendall's observations without success.

METHODS

In every technical procedure Kendall's instructions were followed rigorously. The K medium was prepared as follows:

Fresh hog intestine was opened, cleaned and ground in a meat chopper. The ground material was immersed at once in four volumes of 95 per cent alcohol, and was extracted at 37°C. for two days with occasional stirring. The alcohol was then removed and fresh alcohol added. This was repeated twice, making three extractions in all. The dry tissue residue was then extracted with benzol and the benzol removed by filtration and then by an air current. The dried material was ground to a fine powder in a mortar and was kept in a stoppered container. The actual me-

[1] This investigation was supported by a grant from the John J. Abel Fund for Research on the Common Cold.

dium was prepared by adding 2 per cent by weight of the dried ground intestine to Tyrode solution. Small amounts of $NaHCO_3$ were then added and the final reaction was brought to pH 7.4. The medium was thoroughly mixed and tubed in 10-cc. amounts. Sterilization was accomplished by a single exposure to 15 pounds live steam pressure for twenty minutes in the autoclave.

The preparation of the test substances for cultivation in this medium was as follows: The various strains of typhoid bacilli were cultivated in K medium for forty-eight hours at 30°C., and after dilution with twice the volume of sterile physiological salt solution, were filtered through new Berkefeld V filters and inoculated into fresh K medium and control media. Staphylococcus bacteriophage was obtained by adding small amounts of bacteriophage to young growing cultures of *Staph. aureus* and permitting lysis to take place during an incubation period of eighteen hours at 37°C. The lysed cultures were filtered through new Berkefeld W and Seitz (Uhlenhuth model) filters. The filtrates were used to inoculate both the K medium and the control media. The filtrates of the N.Y.V. strain of the Beta hemolytic streptococcus were prepared in the same manner as the filtrates of the typhoid bacillus cultures. Rhinopharyngeal washings made with Tyrode's solution from individuals ill with early colds and filtered through Berkefeld W candles, were used as the source of the filterable agent of the common cold. A dermal strain of commercial vaccine virus was employed in the cultivation of vaccine virus.

Before inoculation, the sterile K medium was heated in boiling water to drive off the oxygen and then cooled rapidly in cold tap water. Inoculation was always carried out by adding to the K medium 0.5, 0.25 and 0.05 cc. of filtrate, respectively, to 10 cc. of the medium. The K medium cultures were incubated at 30°C. and the cultures were routinely examined for signs of growth at twenty-four, forty-eight and seventy-two hours, as well as at the end of seven and ten days. All cultures were preserved for a period of from three to six weeks before being finally discarded. Sub-cultures upon rabbits' blood agar were made at frequent intervals during the period of observation of the cultures and at the time when the cultures were discarded. Control tubes of

rabbits' blood broth and control rabbits' blood agar plates were inoculated from all filtrates and in every experiment uninoculated tubes of K media were included.

EXPERIMENTAL

Seventeen tests were carried out with three strains of *B. typhosus* (Rawlings, Hopkins and Goggle strains). All 17 tests were carried through one generation, while 10 experiments were carried through the second, and 6 through a third, generation. In no instance was there any evidence of the production of a "filterable" form of the typhoid bacillus with a subsequent transformation of this "filterable" form into a visible typical typhoid microörganism.

Twirty-two series of experiments were made with the Gratia strain of staphylococcus bacteriophage. Half of these tests were made with Seitz filtrates and the other half with Berkefeld W filtrates of the bacteriophage. In each instance 16 experiments were carried through one generation, 6 through two generations and 3 through the third generation of sub-cultures. All filtrates contained bacteriophage (as proved by their ability to bring about lysis in young growing cultures of staphylococci) when inoculated into tubes of K media. Staphylococci were not recovered from the K medium, the controls, or the rabbits' blood agar plate sub-cultures from the various generations of the K medium cultures. The four attempts to produce a filterable form of the N.Y.V. strain of Beta hemolytic streptococcus were likewise unsuccessful.

Five cultivation experiments, each carried through three generations, were made in an effort to recover filterable cocci from K medium inoculated with Berkefeld W filtrates of the rhinopharyngeal washings from individuals ill with common colds. These tests remained without results. Sixteen samples of a commercial strain of dermal vaccine virus were cultivated in the K medium. From 3 of the samples, diphtheroids, and from 3 others, *B. subtilis*, were cultivated in the first generation. Eight tests were carried through the second and third generations respectively without any signs of growth. It is well known that

vaccine virus occasionally contains non-pathogenic microörganisms and no importance was attached to the recovery of diphtheroids and *B. subtilis* from the vaccine virus.

CONCLUSION

Although Kendall's technique was followed minutely, filterable forms of *B. typhosus* and Beta hemolytic streptococci were not produced. It was not possible to cultivate staphylococci from staphylococcus bacteriophage, nor a filterable coccus from "cold" virus. Diphtheroids and *B. subtilis* were cultivated occasionally from commercial vaccine virus, but in view of the well-known observation that these organisms may be contaminants of vaccine virus, no importance was attached to these findings.

REFERENCES

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